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Non-Peptidic, Non-Prenylic Inhibitors of the Prenyl Protein-Specific Protease Rce1

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Abstract—Several compounds designed as bisubstrate analogues of protein farnesyltransferase inhibited the prenyl protein-specific protease Rce1, qualifying them as lead structures for a novel class of non-peptidic, non-prenylic inhibitors of this protease.

Most proteins containing a C-terminal Ca_1a_2X sequence undergo series of posttranslational modifications required for their full biological activity.[1] The first reaction in the series is the transfer of either a 15-carbon farnesyl or a 20-carbon geranylgeranyl isoprenoid lipid from the corresponding isoprenoid diphosphate to the thiol of the cysteine residue of the Ca_1a_2X motif. The a_1a_2-positions are generally occupied by amino acids containing aliphatic side chains, while the amino acid in the X-position determines which of the two isoprenoid lipids will be attached to the protein; those terminating at leucine generally receive a geranylgeranyl residue whereas serine, methionine, glutamine or alanine specifies farnesylation. The second step in the sequence is the endoproteolytic removal of the C-terminal a_1a_2X tripeptide; for most of these proteins this step appears to be mediated by the prenyl protein-specific protease, Rce1.[1c] Finally, the now C-terminal *S*-prenyl cysteine is subject to methylation by the isoprenylcysteine methyltransferase, Icmt (Fig. 1).

The posttranslational modification of Ca_1a_2X-proteins has received much interest during the past years because of the involvement of many of these proteins, particularly the Ras proteins, in the development of human cancer.[1] Research efforts have been focused on the development of inhibitors of protein farnesyltransferase.[2] While the protein prenyltransferases are well-characterized, the prenyl protein-specific protease has only recently been cloned and characterized.[3] Few inhibitors of this enzyme have been described, and these are almost exclusively prenyl peptide-based compounds that function as substrates or substrate mimics.[4] While such peptide-based compounds are valuable research tools, their suitability as potential drugs is limited by their poor cell permeability and inherent instability arising from enzymatic degradation. Furthermore, compounds carrying a prenyl residue are difficult to prepare and are sensitive to oxidation.

We recently described a series of bisubstrate analogue inhibitors of protein farnesyltransferase that lack any peptidic or prenylic structures.[5] These bisubstrate inhibitors are composed of three building blocks: a farnesylmimetic, a linker and an a_1a_2X-peptidomimetic (Fig. 2). These bisubstrate analogues can also be regarded as product analogues of the farnesyltransferase reaction, as they mimic the structure of the farnesylated Ca_1a_2X-peptide. Since this type of farnesylated peptide is also the substrate of the prenyl protein-specific protease, we rationalized that these compounds might be inhibitors of this enzyme also.

Representative bisubstrate analogue farnesyltransferase inhibitors were assayed for their ability to inhibit Rce1 by a recently developed method.[3] Briefly, the inhibitors were incubated with farnesylated Ki-Ras in the presence of membranes containing recombinant Rce1 prenyl protein protease. Following the proteolysis reaction, the proteolyzed proteins were subject to methylation by recombinant Icmt using [^{3}H]AdoMet as a substrate. The amount of processed protein was determined in a filter binding assay.[6]

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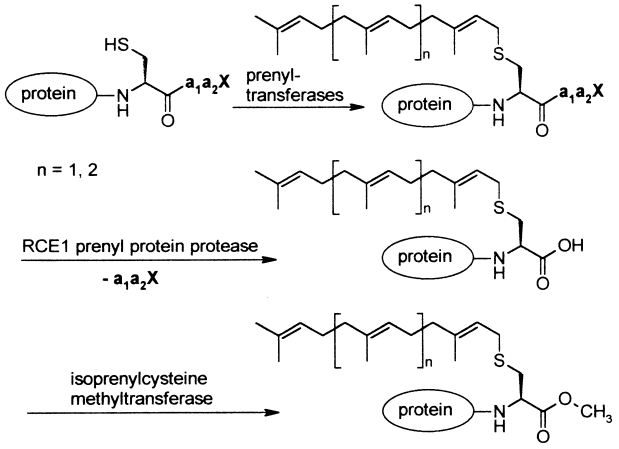


Figure 1. Posttranslational modification of Ca_1a_2X-proteins.

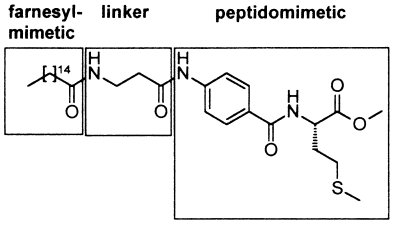
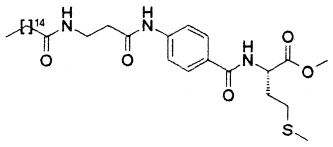
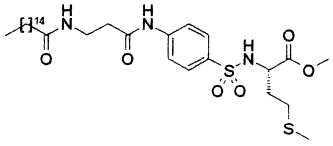
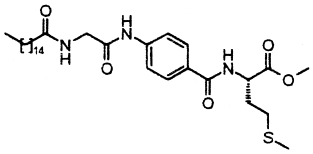
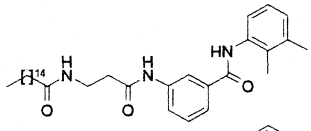
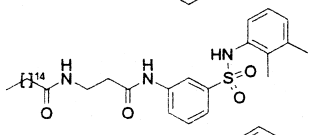
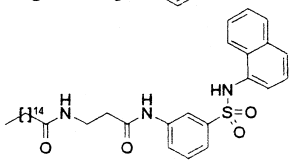
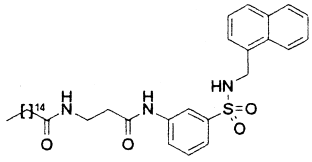
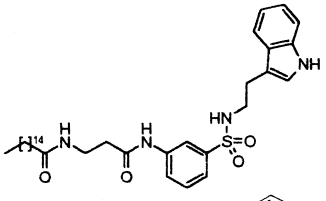
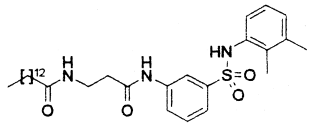
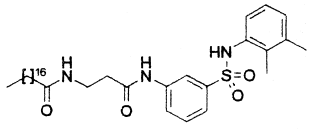
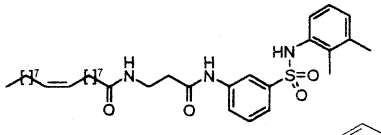
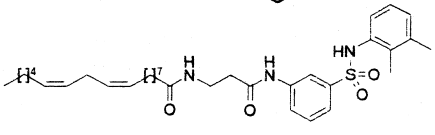


Figure 2. Modularily composed bisubstrate analogue farnesyltransferase inhibitor.

While the majority of the bisubstrate analogue farnesyltransferase inhibitors showed only weak activity as Rce1 inhibitors when tested at a concentration of 10 μM, three compounds (**12**, **14** and **16**) displayed significant inhibitory activity with IC_{50} values in the range of 10 μM, qualifying them as a novel class of non-peptidic, non-prenylic inhibitors of this prenyl protein-specific protease. None of the compounds showed any significant inhibitory activity towards the Imct methyltransferase (data not shown), verifiying that the inhibitory activity observed in the assay was against Rce1.

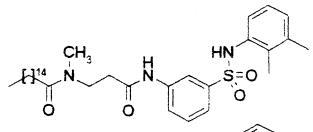
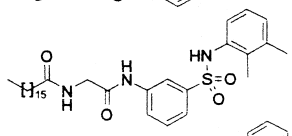
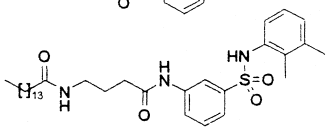
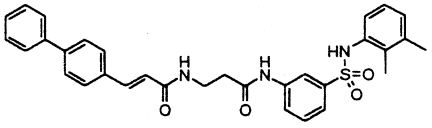
Some structure–activity relationships can be deduced from this series of inhibitors. The bisubstrate analogues can be grouped into two structurally different classes—those such as as compounds **1**–**3** containing an a_1a_2X-mimetic substructure and those (the remaining substances) that lack a structural equivalent of the terminal X-amino acid. Significant activity was found exclusively with the latter group, i.e., bisubstrate analogues carrying 3-amino-*N*-(2,3-dimethylphenyl)benzenesulfonamide as an a_1a_2-mimetic, indicating that the X-amino acid is of minor importance. Inhibitory activity is also dependent on the structure of the linker and the farnesylmimetic; the linkers β-alanine and glycine and the farnesylmimetics linoleoyl and margarinoyl produced active prenylated protein protease inhibitors, as did the aromatic 4-phenylcinnamic acid. The three active Rce1 inhibitors displayed farnesyltransferase inhibitory activity in the same order of magnitude (**12**: IC_{50}(FTase) = 3.8 μM; **14**: IC_{50}(FTase) = 8.5 μM; **16**: IC_{50}(FTase) = 3.4 μM) as Rce1 inhibition. Therefore, selectivity is present in this series

Table 1. Inhibition (Rce1 inhibition at 10 μM or IC_{50}) of Rce1 prenyl protein protease by compounds **1**–**16**

Compound		Rce1 inhib.
1		15 ± 1%
2		28 ± 18%
3		22% ± 2%
4		11 ± 13%
5		18 ± 2%
6		< 5%
7		20 ± 7%
8		23 ± 9%
9		< 5%
10		< 5%
11		< 5%
12		IC_{50} = 14.0 ± 1.3 μM

(*continued on next page*)

Table 1 (*continued*)

Compound		Rce1 inhib.
13		22±1%
14		IC_{50} = 7.0 ± 0.8 μM
15		21 ± 6%
16		IC_{50} = 7.0 ± 5.0 μM

of compounds only in so far as there is selectivity against farnesyltransferase, since there are inhibitors of this enzyme (for example compound **10** (IC_{50} for FTase = 2.5 μM)) which do not inhibit Rce1.

In summary, we have demonstrated a class of non-peptidic, non-prenylic compounds to be inhibitors of the prenyl protein-specific protease Rce1. These inhibitors are structurally more simple, and synthetically more accessible, than the peptidic protease inhibitors described to date. Hence, they could serve as lead structures for further development of a novel class of non-peptidic, non-prenylic inhibitors of this enzyme that could provide utility not only as research tools but also as anti-cancer agents (Table 1).

Acknowledgements

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References and Notes

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6. **Rce1 inhibition assay:** Inhibitors were prepared in DMSO to final concentrations of 1000, 100, 10 and 1 μM. Immediately prior to the assay, the inhibitor solutions were diluted 1:10 with H_2O. Diluted inhibitor (5 μL) or diluted DMSO alone (for control reactions) was added to a 42 μL reaction mixture consisting of 75 mM Hepes, pH 7.5, 5 mM $MgCl_2$, that contained membranes (containing 100 ng total protein) derived from Sf9 cells producing recombinant human Rce1[3]. These mixtures were incubated for 10 min at room temperature and then reactions initiated by the addition of farnesylated K-Ras (final concentration of 3 μM). Reactions were carried out at 37 °C for 10 min. Protease reactions were terminated and methylation reactions initiated by the addition of 20 μL of 3.3 mM $NaPO_4$, pH 7.0, 90 mM EDTA, 300 μM tosylphenyl chloroketone, 10 mM 1,10-phenanthroline, 1 mM phenylmethylsulfonylfluoride, 17.8 μM [^{3}H]AdoMet (7.7 Ci/mmol), and membranes (containing 1 μg protein) derived from Sf9 cells producing recombinant yeast Icmt, Ste14. These reactions were conducted at 37 °C for 20 min, whereupon reactions were terminated and radioactivity incorporated into the farnesyl-Ras determined as described.[3]